NEWS

Whole-genome sequencing unearths new autism mutations

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Surprise diagnosis: Whole-genome sequencing of one family found the same harmful mutation in a son diagnosed with autism and his previously undiagnosed father.

The first sizable study to use whole-genome sequencing to investigate autism has shown its mettle, revealing new mutations and candidate genes for the disorder, according to a report published 11 July in the *American Journal of Human Genetics*[1].

In an effort to unearth genetic variants that other techniques miss, the researchers swept through the complete genomes of 32 children with autism and their families. They were looking for both *de novo*, or spontaneous, mutations as well as rare inherited ones.

“The ability to cull more *de novo* mutations and the ability to cull accumulated inherited gene variants was really the game changer here,” says lead investigator **Stephen Scherer**, professor of medicine at the University of Toronto.

Scherer and his colleagues found damaging mutations in four genes that had never been linked to autism, and in eight suspected and nine known autism genes, including **CACNA1C**, **SCN2A** and **NRXN1**. The study’s sample size is modest, but most other projects of this kind have been **even more limited**, to a single family of four, for example.

In all, the investigators were able to detect an average of 3.2 million variations of single nucleotides

per genome and 800,000 ‘indels,’ or instances in which an insertion and a deletion occur close together.

The researchers detected harmful *de novo* mutations in 15 of the children with autism. They determined that these mutations may contribute to autism symptoms in six of the children, or 19 percent, which is twice the proportion that other methods have turned up.

The researchers also identified 12 inherited mutations in the X chromosome in eight of the children and their mothers, affecting three X-linked genes already associated with autism — **AFF2**, ARHGEF6 and **DMD** — along with three genes considered candidates for autism and one gene not known to be associated with autism.

One child has mutations in both ARHGEF6, a gene associated with intellectual disability, and ZC3H12B, a gene previously linked to autism[2].

Many methods:

The project is an early stage of a **massive effort** to sequence 10,000 whole genomes, funded by the autism science and advocacy organization **Autism Speaks** in partnership with the Chinese genomics company **BGI**. A **similar effort** is underway in the U.K. The approach is meant to offer an alternative to techniques that may be faster and considerably cheaper, but can access only a small portion of the genome.

Multiple other techniques can expose the genome’s relationship with autism, but each has its limitations.

For example, microarrays excel at detecting **copy number variations** (CNVs), large duplications or deletions of DNA[3]. But they can only find CNVs longer than 50 kilobases, encompassing roughly three to five genes. In contrast, whole-genome sequencing can find CNVs in the 100-nucleotide range and captures a much higher proportion of them.

Most sequencing studies in autism have focused on the **exome**, the 1.5 percent of the genome that holds genes known to code for proteins. Exome sequencing can pull out most of the genes within these coding regions for the bargain price of about $1,000 per genome. But it leaves out entire swathes of the genome[4] — including about 10 percent of the exome itself — that might hold valuable information about disease risk.

“There’s always a chance when you only sequence part of the genome [that] the thing you’re looking for will be in the part you don’t look at,” says **Michael E. Zwick**, associate professor of human genetics at Emory University in Atlanta, Georgia, who was not involved in the study. “So the solution is to look at everything.”

Cost is whole-genome sequencing's Achilles' heel: Decoding a single genome in a research trial that is subsidized by a sequencing company can run as little as $3,000 each, but the standard price edges closer to $6,500.

"Whole-genome sequencing is at least as good as exome sequencing, of course, but is it worth the extra expense?" asks **Michael Wigler**, professor of genetics at Cold Spring Harbor Laboratory in New York. Still, Wigler adds, "In time it will be as cheap as exome sequencing, because **sequence costs** are dropping, yet exome-capture costs are fixed and substantial."

Combing through the genomes, the researchers uncovered surprises in some families. For example, a boy with both autism and intellectual disability has rare inherited mutations in both the X-linked gene AFF2 from his mother — a rare variant of which is associated with **fragile X syndrome** — and the KCNQ2 gene from his father, which is associated with benign seizures in infancy. Defects in the latter gene are suspected in intellectual disability associated with autism.

The father had had seizures as a baby but was not diagnosed with autism until after the study, and the child's sister, who carries the same rare mutation, displays some autism-like behaviors. This suggests that mutations in KCNQ2 play a role in autism.

Scherer and his colleagues also found mutations linked to other disorders, such asKallmann syndrome, a rare disorder that has not previously been associated with autism. "These parents go on diagnostic odysseys trying to explain not just autism, but all of the other symptoms their children have," says Scherer.

For example, the researchers found a missense mutation in the DMD gene, located on the X chromosome, in a 14-year-old boy with autism. This mutation is known to cause a form of muscular dystrophy. The boy does not yet show any signs of the disorder, but can now be monitored for it.

The next step will be to find out the function of the genes disrupted by the identified mutations, which can be time-consuming and arduous, says Zwick.

There may also be a problem of diminishing returns on further study of the 3,000 or so more interesting variants, many of them newly identified. "More than half of the variants will be in less than 1 percent of the population," says Zwick. "When something is exceptionally rare, it is very hard to figure out statistically."

References:

1: Jiang Y.H. *et al. Am. J. Hum. Genet*. Epub ahead of print(2013) **PubMed**

2: O'Roak, B.J. *et al. Nature* **485**, 246-250 (2012) **PubMed**

3: Prasad A. *et al. G3* **2**,1665-1685 (2012) **PubMed**

4: Steinberg K.M. *et al. Mol. Autism* **3**, 8 (2012) **PubMed**